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Research Article

Welcome to Hotel Hymenoptera: monitoring cavity-nesting bee and wasp distribution and their trophic interactions using community science and metabarcoding

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Abstract

Essential ecosystem services are provided by many interactions, including plant-pollinator, predator-prey, and host-parasitoid. These services support food and natural systems through pollination and pest control, however they are challenging to qualify, and previous observational studies may underestimate their complexity. The cavity nesting Hymenoptera are a good example showing all these three interactions and they can be monitored using trap nests.

For this study, trap nests were installed at schools across Canada by community scientists to investigate cavity-nesting bee and wasp distributions and interactions. DNA metabarcoding was used to identify the occupants and their food sources. New bee and wasp distributions were found that might be the result of previous under-sampling or recent range expansions. Detailed bipartite and tripartite networks describing species interactions suggest some novel bee, wasp, and parasite associations. These results encourage further investigation into these interactions using molecular methods as detailed range maps and networks provide information to natural historians and conservationists alike.

Key words: Land use, pollination, range expansion, urban ecology

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Introduction

Ecosystems that are influenced by human development are often highly disrupted and host reduced levels of biodiversity (Elmqvist et al. 2003; Sánchez-Bayo and Wyckhuys 2019). Although many factors contribute to species declines and extinctions, anthropogenically-driven landscape changes are seen as the largest drivers (Vanbergen and Initiative 2013; Sánchez-Bayo and Wyckhuys 2019). Both agricultural intensification and urbanization are landcover changes that alter natural habitats, both reducing and fragmenting the amount of accessible living space for many species. Unfortunately, the extent of these disturbed ecosystems is increasing and has been globally for decades. These disruptions have negative consequences on many of the

ecosystem services we humans rely on, by not only disturbing the species but also their interactions with each other.

An additional consequence of increased urbanization is that it may benefit the establishment and persistence of non-native species over native species (Hernandez et al. 2009; Fitch et al. 2019; Kammerer et al. 2021; Gruver and Caradonna 2021). Non-native bees, for instance, are not only resilient against increasing levels of urban development, but they might benefit from it to a point of outcompeting native species (Fitch et al. 2019). Non-native species are capable of outcompeting native specialist bees since they are generalists, able to feed on a wide variety of food sources (Russo 2016; Fitch et al. 2019; Geslin et al. 2020). Globally, 16 non-native bee species have been purposefully introduced for agricultural purposes, including the European honeybee *Apis mellifera*, multiple bumblebee (*Bombus*) species, and some solitary bees including *Megachile rotundata* (Pitts-Singer and Cane 2011; Graystock et al. 2016; Russo et al. 2021). Additionally, more than 65 non-native species were introduced unknowingly, either mistaken for a similar looking species or transported by accident (LeCroy et al. 2020). The majority (70%) of introduced bee species are cavity-nesting (Russo 2016), increasing the potential for competition with native cavity-nesting bees. Whether they were introduced purposefully or by accident, non-native bee species have the potential to harm native species due to their increased fitness in urban areas.

Solitary cavity-nesting Hymenoptera represent a unique community where multiple ecological guilds occur and interact in the form of bees (pollinators), wasps (predators and pollinators) and their prey, as well as parasitoids which target all the former (Farzan et al. 2017). Reproductive solitary cavity-nesting bee and wasp females occupy their own nests, within hollow, above-ground nesting sites such as pithy stems and wood-feeding beetle burrows (Krombein 1967), and construct linear brood cells from the rear of the tubular nest hole to the front (Farzan et al. 2017; Macivor 2017). In non-parasitic species, each brood cell is separated by species-specific material such as leaves, soil, or tree sap and supplied with food. Cavity-nesting bees provision offspring with a combination of pollen and nectar called bee bread, while wasps provision their nests with arthropod prey, such as spiders, caterpillars, or aphids (Krombein 1967; Farzan et al. 2017; Hoffmann et al. 2018). Both cavity-nesting bees and wasps are hosts for parasitoid wasps which can lay their eggs within the previously constructed nests, either once the nest is completed or while the female nest provisioner is out foraging (Farzan et al. 2017; Osorio-Canadas et al. 2018). Many parasitoid wasps can alternatively inject their egg directly into the host larvae.

While bees are well known for their pollinating services (Klein et al. 2007; Potts et al. 2016), their close relatives, wasps, also provide an additional beneficial ecosystem service. Many wasps act as natural predators that can be used in integrative pest management (IPM) strategies to control insect pests on plants without the use of chemical insecticides (Harris 1994; Hoffmann et al. 2018; Da Rocha-Filho et al. 2020; Brock et al. 2021). Predatory wasps consume nectar and pollen as adults but require arthropods, such as caterpillars or spiders, to provision their offspring (Hoffmann et al. 2018; Wang et al. 2019). In addition to biocontrol agents, wasps are considered important biodiversity indicators as they require multiple ecosystem resources and are sensitive to their loss (Turčinavičiene et al. 2014; Hoffmann et al. 2018). As higher trophic level

organisms, wasps are more vulnerable to habitat change, making conservation strategies increasingly important (da Rocha-Filho et al. 2020).

Due to their nesting habits, cavity-nesting bees and wasps can be sampled in human-made nests. Human-made trap nests, also called bee hotels or nest boxes, mimic natural cavity-nesting sites of hollow plant stems and other holes (Staab et al. 2018). Trap nests may be made from bundled plant stems, holes drilled in wood, paper-based tubes, or a variety of other methods (MacIvor 2017). The first recorded use of trap nests to study cavity-nesting Hymenoptera was by Jean-Henri Fabre (Fabre 1914), and since then, many have used this strategy to study the natural history or ecosystem service potential of cavity-nesting species (Tasei et al. 1976; Bosch 1994; Aguiar and Garófalo 2004). Although trap nests are limited to detecting only solitary cavity-nesting species, they collect additional information on the ecology of these species. Unlike many other passive insect sampling strategies, such as pan traps and blue vane traps, which may detect species in the area, trap nests provide an indisputable way of knowing a species' nesting location (Westphal et al. 2008; Staab et al. 2018). Trap nests are an extremely valuable method of monitoring interactions between species, such as parasitic relationships and plant-pollinator interactions (Staab et al. 2018). The pollen or prey stored as food for larvae in trap nests can be directly linked to the species of bee or wasp that collected it (Staab et al. 2018). This makes the trap nest strategy ideal for studying the interactions of solitary cavity-nesting bees and wasps, their food and prey, and their parasitoids. Although cavity-nesting bees represent only a small fraction of all solitary bee species, their abundance is highly correlated with the overall abundance of nest-provisioning bees (Staab et al. 2018; Mayr et al. 2020). Therefore, they are representative of local bee communities and can allow some extrapolation on how local conditions are affecting bee communities.

Many methods exist to study species interactions and movement; one more recently introduced is DNA barcoding. DNA barcoding is a molecular method of species identification that has been used for over two decades (Hebert et al. 2003). DNA metabarcoding uses a similar premise but identifies multiple species in a community sample (Cristescu 2014). Many studies have used DNA barcoding techniques to identify both insect and pollen specimens (for example Magnacca and Brown 2012; Bell et al. 2016; Bell et al. 2017; Pornon et al. 2017; Gresty et al. 2018). In many insect taxa, taxonomic identification to species level is impossible due to cryptic diversity or incomplete specimens (Packer et al. 2009). The identification of pollen through DNA barcoding is popular since pollen is traditionally very difficult to identify using comparative morphological approaches for taxonomic identifications (Bell et al. 2016; Peel et al. 2019). Recently, DNA metabarcoding has been used to form plant-pollinator networks by collecting pollen from bees' legs (Bell et al. 2017; Gresty et al. 2018). As DNA barcoding databases, such as the Barcode of Life Database (BOLD) and GenBank, continue to grow, more species level identifications become possible using this molecular technique.

To date, only one other study has used DNA barcoding of cavity-nesting bees and wasps and their pollen and prey, respectively, together (Dürrbaum et al. 2023). Our study uses a similar approach to investigate the interactions between solitary cavity nesting Hymenoptera across Canada. Combining a community science approach with DNA metabarcoding, we took an in-depth look at solitary cavity-nesting bee and wasp distributions and interactions. Specifically,

we wanted to determine: 1) What are the distributions of solitary cavity-nesting bee species across Canada and what flowers are they using as sources of nectar and pollen to provision their offspring? (2) What are the distributions of solitary cavity-nesting wasp species across Canada and how are they interacting with their diverse prey and parasitoids? (3) How does local landcover (within 500 m of the trap nest) affect the distribution of solitary cavity-nesting bees and wasps?

Methods

Sample collection

Trap nests were constructed following a design that has been used successfully in past studies (MacIvor and Salehi 2014; MacIvor et al. 2014; MacIvor 2016 – Suppl. material 1: fig. S1). A 30- cm section of 10 cm-diameter PVC pipe was used as housing, with one end cut on a 60° angle to provide weather protection. The other end of the pipe was covered with a PVC pipe cap. A 10 cm diameter circular piece of Styrofoam was cut to fit into the PVC pipe. Ten bee paper tubes (Custom Paper Company, US) for each of three diameters (3.4 mm, 5.6 mm, 7.6 mm diameter) were inserted into the Styrofoam piece after one tube end had been plugged with paper clay. Each trap contained a total of 30 paper tubes. Each tube represented a sample unit, resulting in multiple samples available for a single trap.

During the summers of 2019 and 2020, trap nests were distributed to elementary and high schools across Canada as part of a community science program (beesatschools.ca). In 2019, 88 trap nests were sent out and 42 were returned, and in 2020, 202 nests were distributed and 170 returned to Guelph (Fig. 1). Trap nests were installed from early May to late August to allow phenological coverage for various nesting strategies and provide sufficient observation time for students. Teachers were instructed to install nests facing south at a height of 1–1.5 metres above the ground. They reported the orientation, height, latitude, and longitude of the trap nest, as well as provided photos of the installation to ensure correct use.

Sample processing

Sample extraction

Once nests were returned in late August-early September, cardboard nesting tubes were removed from the Styrofoam and stored at -20 °C until further processing. Tubes were opened using a sterilized knife and forceps, and insect larvae, pollen, and prey were separated from leafy nesting material into sterile tubes. Larvae samples were split into subsamples when necessary to ensure approximately equal tissue mass per DNA extraction. Leaf material was stored for further projects but not used for this study. The diameter of cardboard tube, type of nesting material (leaf, soil, tree sap, etc.), and number of larvae per tube were recorded.

Molecular identification

All molecular lab work was conducted in a clean-room laboratory at the University of Guelph compliant with DNA processing standards. Work surfaces were cleaned with 1% bleach and 70% ethanol, followed by UVC-light radiation

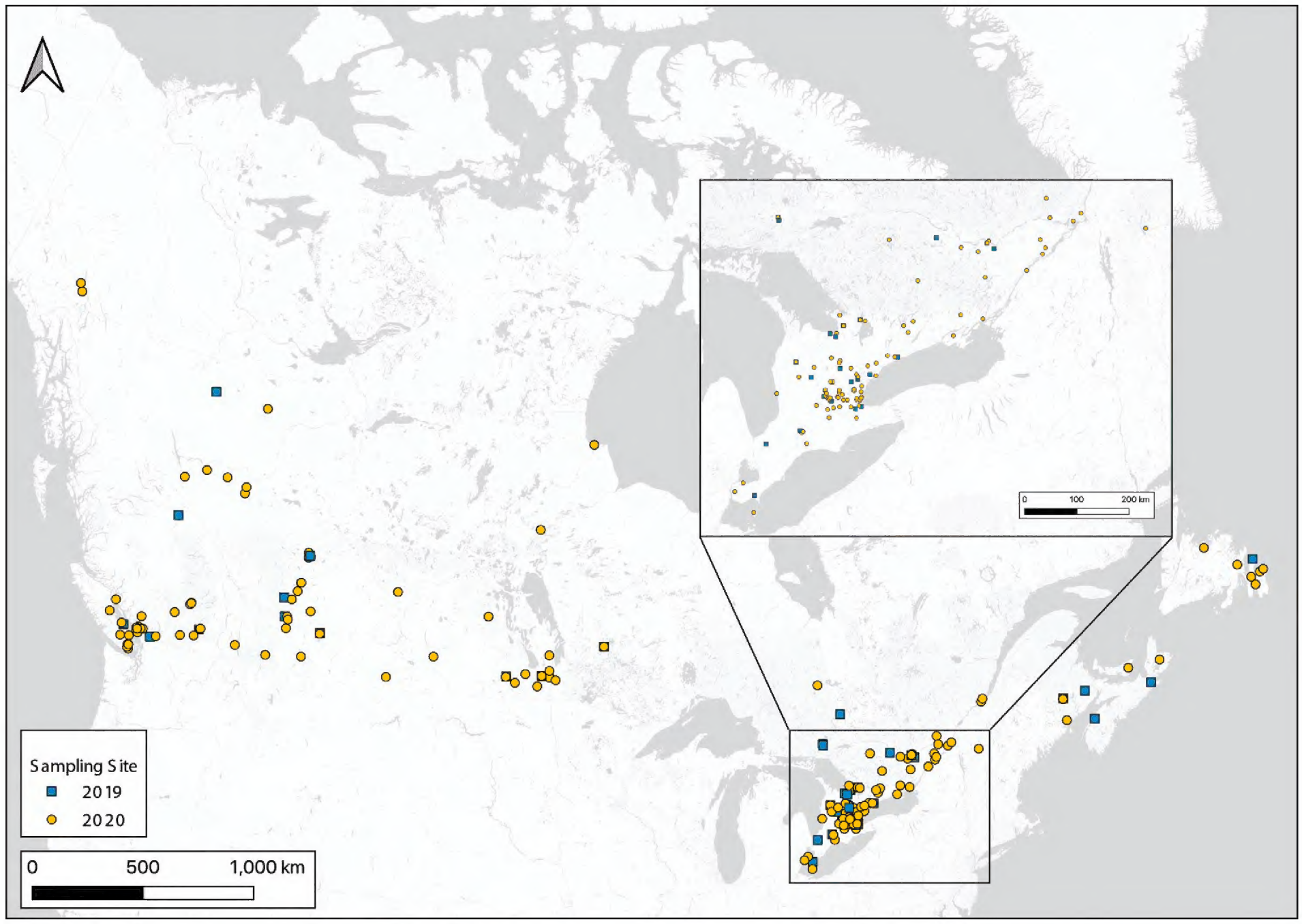

Figure 1. Map showing sampling sites across Canada in 2019 (42 sites, blue) and 2020 (170 sites, yellow).

for 15 minutes. Samples were mechanically masticated using a disposable pestle (VWR International, LLC., PA, USA) to break open the larval cuticle and release pollen. 40–100 mesh sand (ACRO Organics, Thermo Fisher Scientific Inc., MA, USA) and two 5 mm stainless steel beads (IKA Works Inc., NC, USA) were added to further mechanically break-down tissue along with 900 µL insect lysis buffer (700 mM GuSCN, 30 mM EDTA, 30 mM Tris-HCL, 0.5% Triton X-100, 5% Tween-20, pH 8) and 100 µL Proteinase K (QIAGEN, 20 mg/mL). Samples were shaken on a QIAGEN TissueLyser II at 28Hz for two minutes to further pulverize pollen. Samples were incubated on a rocking platform (OHAUS Corporation, NJ, USA) for 2.5 hours at 56 °C, followed by one hour at 65 °C. The resulting lysate was combined with binding buffer (6M GuSCN, 20 mM EDTA, 10 mM Tris-HCl, 4% Triton X-100) in a 2:1 ratio of buffer to lysate, mixed briefly by vortexing, and centrifuged at 1,000 × g for 20 seconds. Lysate was transferred to a glass fibre column (Qiagen, Blood and tissue kit) in three aliquots of 600 µL, centrifuged at 10,000 × g for two minutes between aliquots. 300 µL of binding mix (150 µL binding buffer, 150 µL 96% ethanol) was added to the column followed by centrifuging at 10,000 × g for two minutes. The column was washed twice with 600 µL of wash buffer (60% EtOH, 50 mM NaCl, 10 mM Tris-HCl, 0.5 mM EDTA) and centrifuged at 10,000 × g for two minutes between washes. The column was then centrifuged at 10,000 × g for two minutes to dry. The glass fibre column was transferred to a clean 1.5 mL microcentrifuge tube and 25 µL of elution buffer (0.1M Tris-HCL, pH 8) was added. The sample was incubated at 56 °C for one minute then centrifuged at 10,000 × g for two minutes.

This was done twice for a total elution volume of 50 µL. Each group of extractions contained one extraction negative control.

Gene fragments were amplified using a PCR reaction mix of 12.5 µL of QIAGEN multiplex plus master mix, 1.25 µL each of 10X PCR forward and reverse primer, 5 µL of DNA template, and 5 µL of nfH20. For bee, wasp, and prey identification, the BF3 + BR2 primer set was used (Suppl. material 1: table S1; Elbrecht et al. 2019) to isolate the mitochondrial cytochrome c oxidase I (COI) gene. For plant identification, the rbcl1 + rbcLB primer set was used to isolate the ribulose bisphosphate carboxylase large chain (rbcL) gene (Suppl. material 1: table S1; Palmieri et al. 2009; Little 2014). The following conditions were used for PCR: denaturation for 5 minutes at 95 °C; 25 cycles of 30 s at 95 °C, 45 s at 50 °C, and 50 s at 72 °C; final elongation for 5 min at 72 °C; then held at 4 °C. Samples were then indexed with custom tags (Suppl. material 1: table S2; Elbrecht and Steinke 2019) during a second PCR utilizing the same thermocycler protocol and the same reaction mix as for the first PCR. Gel visualizations of tagged PCR products were done using 1% agarose gels on GelRed® Nucleic Acid Gel Stain (Biotium, CA, USA). PCR products were normalized using the SequalPrep™ Normalization Plate Kit (Invitrogen, Thermo Fisher Scientific Inc., MA, USA). Samples were pooled and the final library was cleaned using left-sided size selection with 0.7X SPRISelect (Beckman Coulter, CA, USA). Libraries were quantified using the Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific Inc., MA, USA). Libraries from 2019 were sequenced using an Illumina MiSeq (v3 kit) (2 × 300 bp) at the University of Guelph Advanced Analytics Centre's Genomics Facility. Libraries from 2020 were sequenced using one lane on a SP Chip on Illumina Novaseq 6000 (v1.5 kit, 2 × 250 bp) at Sick Kids Hospital's DNA Sequencing and Synthesis Facility.

Data analysis

Data processing

Raw sequence data quality control was initially done using FastQC v0.11.8. Resulting libraries were analyzed using JAMP v0.69 (github.com/VascoElbrecht/JAMP). Sequences first underwent demultiplexing, then paired-end merging using Usearch v11.0.667 with fastq_pctid=75 (Edgar 2010). Primer sequences were trimmed using Cutadapt v1.18 with default settings (Martin 2011) and only sequences where primers could be trimmed at each end were kept. Additionally, Cutadapt removed sequences outside lengths of 421 +/- 10 bp for COI or 184 +/- 10 bp for rbcL. Poor quality sequences were removed in Usearch with an expected error value of 1 (Edgar and Flyvbjerg 2015). Filtered reads for each sample were dereplicated and singletons removed. Each amplicon sequence variant (ASV) of COI was compared to two custom reference databases, one for Hymenoptera specifically, and one for insects in general. rbcL ASVs were run against a plant reference. Both the Hymenoptera and insect databases consisted of downloads of the Barcode of Life Datasystems (BOLD - www.boldsystems.org; Ratnasingham and Hebert 2007). The custom plant database was assembled using sequences obtained from NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank; Sayers et al. 2018). Read counts for any OTU recovered from negative controls on a plate were subtracted from the counts for the same OTU in the non-control wells in the run. When this subtraction reduced the read count for an OTU to zero, its

occurrence was removed. This step was meant to reduce the effects of rare tag switching (Elbrecht and Steinke 2019) as well as background contamination.

Insect detections (using COI) from 2019 and 2020 with less than 50 and 90 reads respectively were removed for all analyses, representing 85% of all detections. The read threshold was increased in 2020 due to the increased detection sensitivity of Illumina Novaseq compared to Illumina Miseq. Hymenoptera detections with fewer than 1,000 reads (94% of all detections) were removed from interaction analyses. Such minimum sequence copy thresholds are one adaptable method commonly used to reduce the prevalence of artefacts (Hänfling et al. 2016) and we chose rather high values to ensure reads represent true nesting occupants and not accidental visitors or other non-target taxa.

Statistical analyses

Unless otherwise mentioned, all statistical analyses were done using R statistical software v4.1.0 (R Core Team 2021).

Bipartite plant-pollinator and predator-prey networks were created using the R *bipartite* package (Dormann et al. 2008). Gephi was used to create a tripartite network (Bastian et al. 2009). Prior to creating networks, individual species were investigated to determine various life history traits. Insects were categorized based on trophic function as prey, pollinator, predator, or parasitoid (Krombein 1967; Krombein and Hurd 1979; Cane et al. 2007; Sheffield et al. 2011). Historical ranges in the literature were used to categorize each species as native or non-native. For taxa that could not be identified to species, a genus or family level identification was used. For these taxa, native range could not be identified, and in some cases neither could trophic function. These were subsequently left out of these analyses. Plants were analyzed at a minimum genus level since species-level identifications using rbcL have variable reliability (Braukmann et al. 2017). Network metrics including nestedness, links per bee species, and connectance were calculated using *bipartite*. Nestedness measures whether locations with low species richness are unique from locations with high species richness, and connectance is the proportion of possible links among species that are seen. Species range maps were made using historical data taken from GBIF using the R *rgbif* package (Chamberlain et al. 2022).

Spatial analyses were done using QGIS v3.18.3 (QGIS Development Team 2021). Local landscape features were determined by mapping site locations alongside the 2015 Land Cover of Canada dataset (Natural Resources Canada 2015). A 500-meter buffer was created for each site, then QGIS's zonal histogram tool determined the number of pixels per buffer of each landcover type (nine types of forest, wetland, cropland, barren land, urban, water, snow/ice). These were converted into percent cover, and finally prominent landcover type was determined by which landcover type had the greatest area. The nine types of forest cover were amalgamated into one forest designation. Once landcovers were determined, redundancy discriminant analyses (RDAs) were run to investigate the relationship between community composition and land cover designation using the vegan package in R (Oksanen et al. 2008). The vegan package was also used to calculate bee and wasp community richness and abundance (Oksanen et al. 2008). Analyses of variance (ANOVAs) were run to determine whether communities were significantly different based on landcover types.

Results

General results

High quality DNA was extracted from 887 samples corresponding to 638 cardboard tubes (Table 1). However, only 52% of 2019 tubes were successfully sequenced compared with 91% of 2020 tubes. Reads counts pre- and post-filtering for all Illumina sequencing runs can be found in Table 2. Raw data are available on NCBI SRA under the accession number PRJNA1232610.

Sequences revealed 2,313 hymenopteran detections from 78 taxa. These included 29 species of bees (24 cavity-nesting, 5 cleptoparasitic; Fig. 2A, Suppl. material 1: table S3) and 49 taxa of wasps (27 predatory, 22 parasitic/parasitoid; Fig. 2B). Species richness and abundance of bees and wasps were significantly higher in 2020 than in 2019 (ANOVAs: bee abundance $p = 0.01$, bee richness $p < 0.01$, wasp abundance $p < 0.01$, wasp richness $p < 0.01$). In 2020, 25 species of bee and 41 species of wasp were found, compared to 7 and 16 in 2019. There was considerable overlap between years, with 85% of species found in 2019 also found in 2020 (Fig. 2). A further break-down of bee and wasp diversity as well as species richness variation between nests can be seen in Suppl. material 1: fig. S2. Analysis of plant tissue revealed 384 plant genera from 91 families and 36 orders (Suppl. material 1: fig. S3). A full list of all identified genera can be found in the Suppl. material 1: table S4.

In addition to hymenopteran occupants, 328 detections from 11 other insect orders were found. Twelve taxa of parasitic/parasitoid insects were detected, including 10 Diptera and one each of Coleoptera and Strepsiptera. In addition, 72 taxa of potential prey were detected, including 10 Araneae, 10 Coleoptera, five Diptera, one Ephemeroptera, eight Hemiptera, 27 Lepidoptera, seven Orthoptera, and four Thysanoptera species. Three additional taxa (*Forficula auricularia* (Dermaptera), *Trogoderma inclusum* (Coleoptera), *Elipsocus moebiusi* (Psocoptera)) are thought to be incidental detections. Literature does not classify them as parasites, predators, pollinators, or prey, and all are opportunistic in determining their nest. For one taxon (*Megaselia* sp. (Diptera)) we were unable to determine whether we obtained a parasitic species, a scavenger or prey given the lack of a species designation.

Table 1. Trap nest installation, occupation, and extraction in 2019 and 2020.

Year	Nests installed	Nests occupied	Tubes occupied	Min / max tubes per site	Average tube occupancy per nest	Nests sequenced	Tubes sequenced
2019	44	22	172	1 / 24	7.8 +/- 1.4	20	90
2020	170	93	604	1 / 27	6.5 +/- 0.6	93	548

Table 2. Trap nest sequencing reads generated by Illumina sequencing prior to and post filtering for COI and rbcL in both sampling years (2019 and 2020).

Year	Marker	Unfiltered Reads	Average Reads Post-Filtering per Sample	Total Reads Post-Filtering
2019	COI	22,048,123	49,290	7,097,815
	rbcL	17,179,971	56,982	8,091,722
2020	COI	574,568,330	75,612	57,842,823
	rbcL	514,959,887	67,969	51,996,248

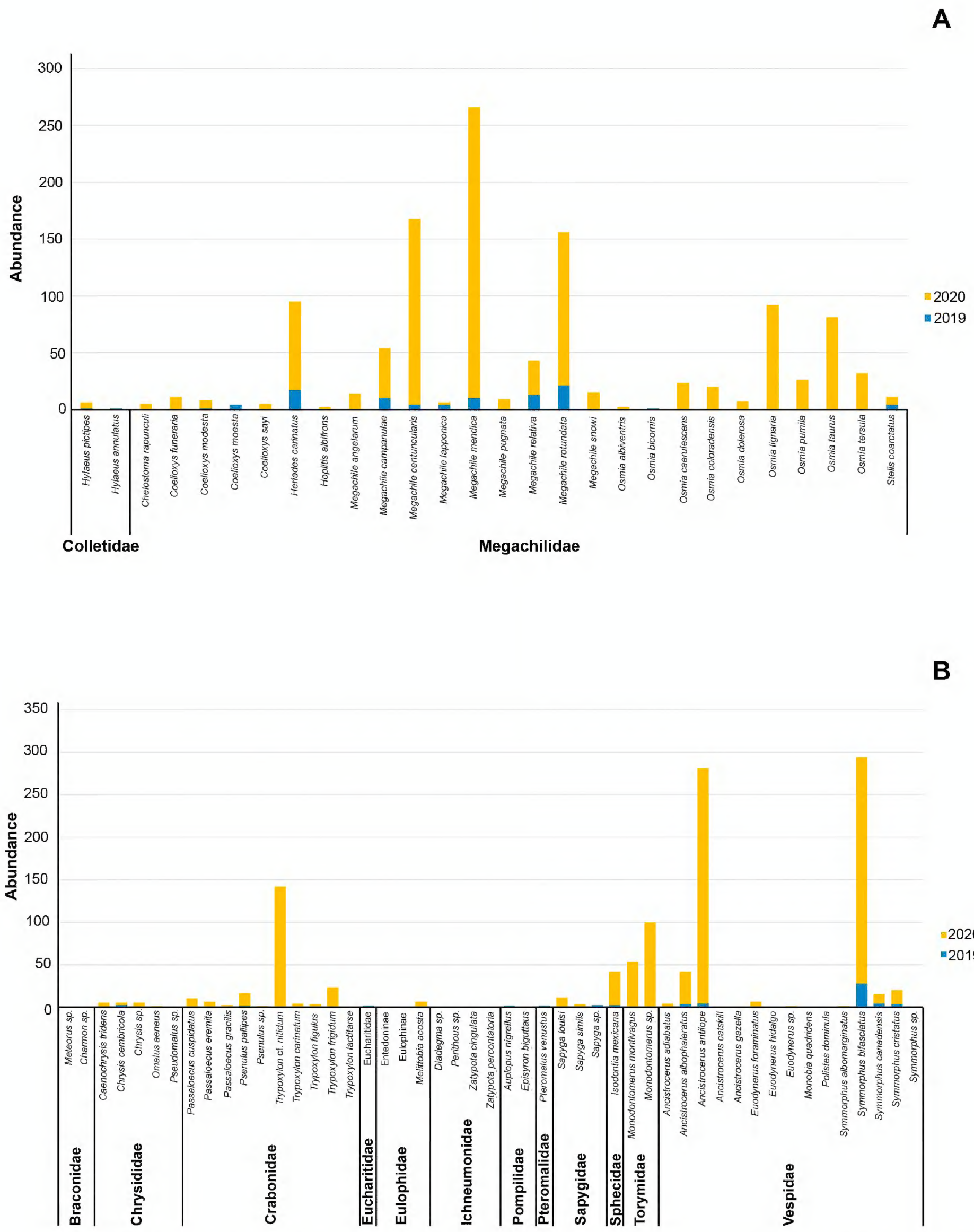

Figure 2. Bee (**A**) and wasp (**B**) abundance by species and family in trap nests installed across Canada in 2019 and 2020 detected through COI metabarcoding.

Distribution

When compared to previous distributional records (accessed from GBIF and discoverlife.org), 10 bee species appear to have expanded ranges, including three non-native species (*Chelostoma rapunculi*, *Hylæus pictipes*, *Osmia*

taurus) and seven native species (*Coelioxys modesta*, *Megachile campanulæ*, *Megachile angelarum*, *Megachile snowi*, *Megachile mendica*, *Osmia dolerosa*, *Osmia cærulescens*). Distribution maps for these species are shown in Suppl. material 1: fig. S3, while maps of the 17 species with ranges comparable to existing records can be found in Suppl. material 1: fig. S4. Four species of wasps were found to have potentially expanded ranges (*Passaloecus eremita*, *Passaloecus gracilis*, *Psenulus pallipes*, *Trypoxylon* cf. *nitidum* – Suppl. material 1: fig. S5).

Interactions

A food network including all natural enemies, predatory wasps, pollinators, plants, and prey demonstrates the overall complexity of species interactions detected (Fig. 3). A bipartite network for the 22 species of cavity-nesting bees and 88 plant families showed patterns consistent with polylectic pollen consumption: i.e. each bee species harvested pollen from a variety of plant genera

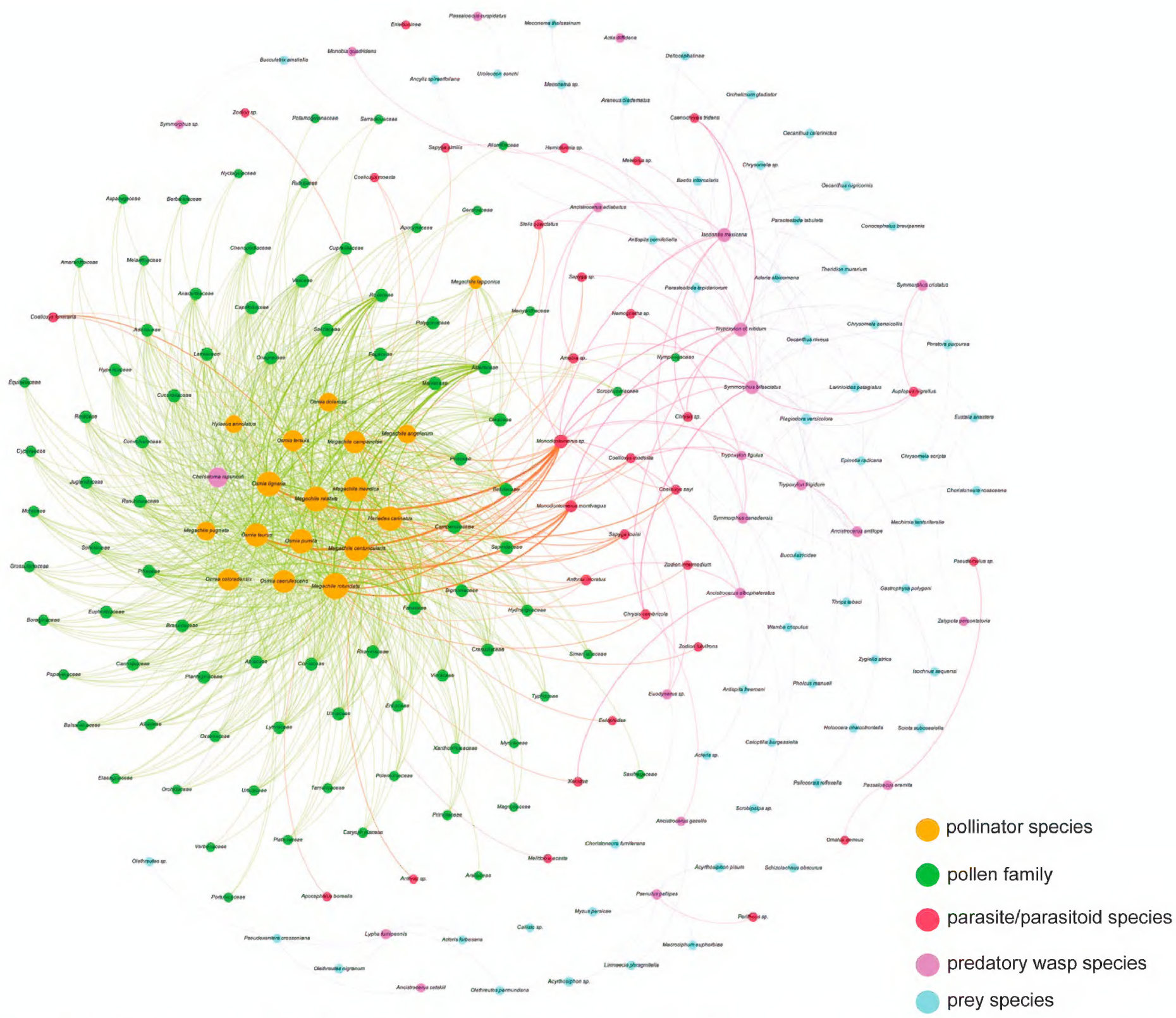

Figure 3. Multipartite network showing interactions between parasites (red), predatory wasps (pink), pollinators (orange), insect prey (blue), and plants (green). The diameter of the circles represents species abundance, and the thickness of a connecting line shows interaction strength.

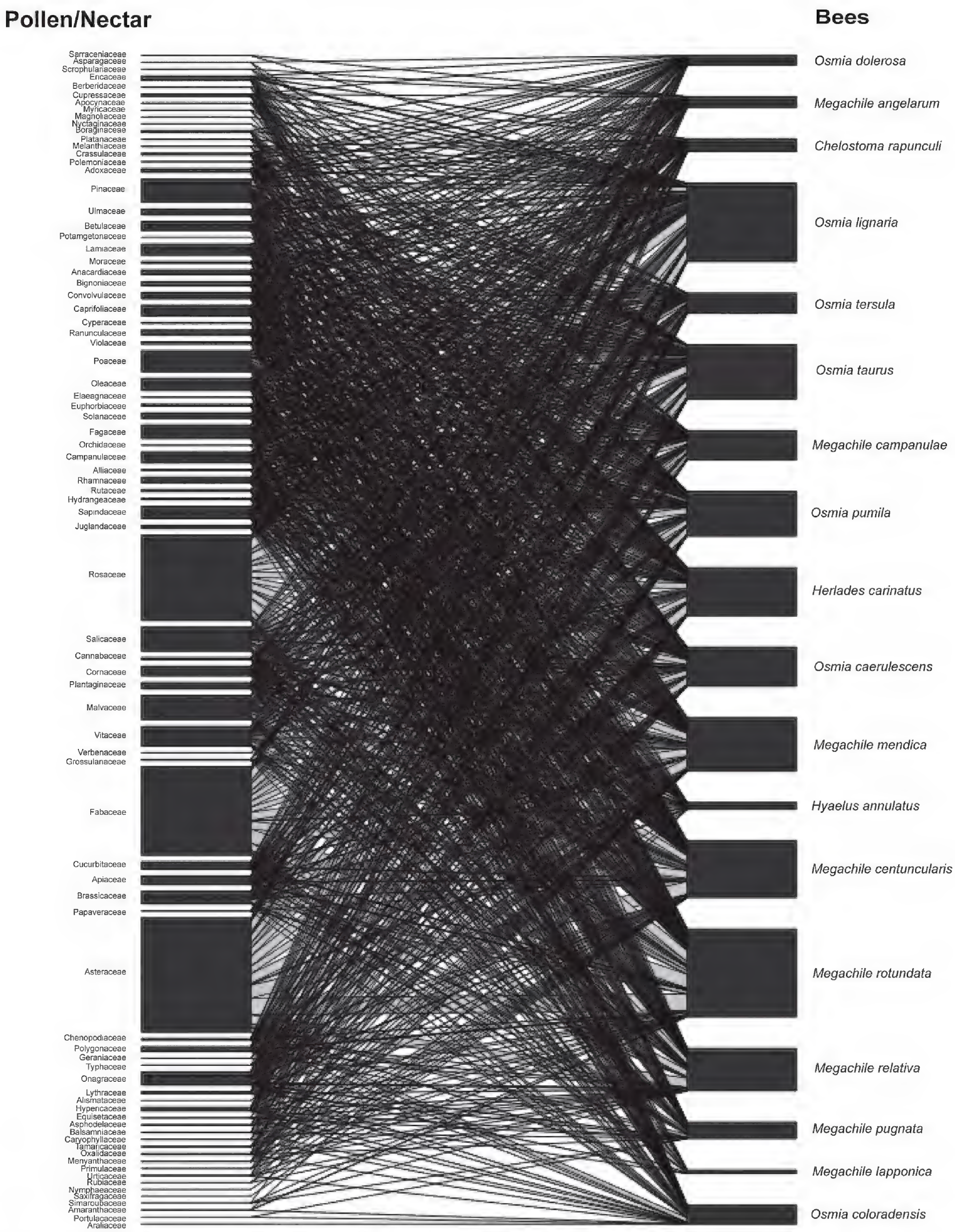

Figure 4. Bipartite network of all pollen-collecting bees found with pollen/nectar in trap nests in 2019 and 2020.

and families (Fig. 4). The number of unique plant families and genera visited by each species at crop, forest and urban sites is shown in Suppl. material 1: table S5. Polylectic foraging agrees with historical foraging records (Suppl.

material 1: table S6) for all but three bee species (*Chelostoma rapunculi*, *Megachile lapponica*, *Megachile pugnata*) that were considered oligolectic (Gathmann and Tscharntke 2002; Sheffield et al. 2011). Three out of four *C. rapunculi*, two out of four *M. lapponica*, and one out of seven *M. pugnata* were detected in nests with their known host plant, in addition to many other plant genera. Most interactions with bees involved three plant families: Asteraceae (20%), Fabaceae (13%), and Rosaceae (13%). Nestedness of the overall bipartite plant-pollinator network was low at the plant family level (19.7), showing that interactions at sites with low species richness were not generally a subset of interactions at sites with high species richness. At the plant family level, the overall network showed an average of 8.7 links per bee species and a connectance of 0.59. The urban network demonstrated the highest number of pollinators (18) and plants (77), links per species (8.3), connectance (0.57), nestedness (19), and cluster coefficient (0.6) of the landscape-based networks. Full details of all plant-pollinator network metrics can be found in Suppl. material 1: table S7.

Predatory wasps were analyzed in bipartite networks to evaluate their interactions with prey (Fig. 5). Eleven of these species were identified in cavities with prey that is not prominent in historical records. All predatory interactions can be seen in Suppl. material 1: table S8.

Trap nests from 59 sites (49%) and 152 cardboard tubes (27%) were found to contain natural enemies (parasites/parasitoids) and their host(s). The percentage of tubes parasitized per site ranged from nine to 100%. Of all tubes, 30 had multiple natural enemy detections. Parasitic and parasitoid wasps, flies, and beetles were analyzed in bipartite networks to evaluate their interactions with hosts (Fig. 6). Ten natural enemies were identified in cavities with hosts not prominent in historical records. All parasitic interactions are shown in Suppl. material 1: table S9. Overall, 45% of tubes (284) were found with multiple (two to four) Hymenoptera occupants. Often these were hymenopteran parasites/parasitoids along with their hosts, but once those instances were removed, 155 cases remained where multiple predators and/or pollinators nested in the same tube (Table 3, Suppl. material 1: table S10).

Landcover

Landcover was examined around each site using a designation based on which type (crop, forest, or urban) covered the largest percentage of land within a

Table 3. Nest sharing occupant combinations in nest boxes in 2019 and 2020.

Occupant combination	Number of tubes
Two pollinators	59
Two predators	18
One pollinator/one predator	43
Two pollinators/one predator	13
Three pollinators	7
Three predators	2
Two pollinators/two predators	2
Three pollinators/one predators	1
Four pollinators	1

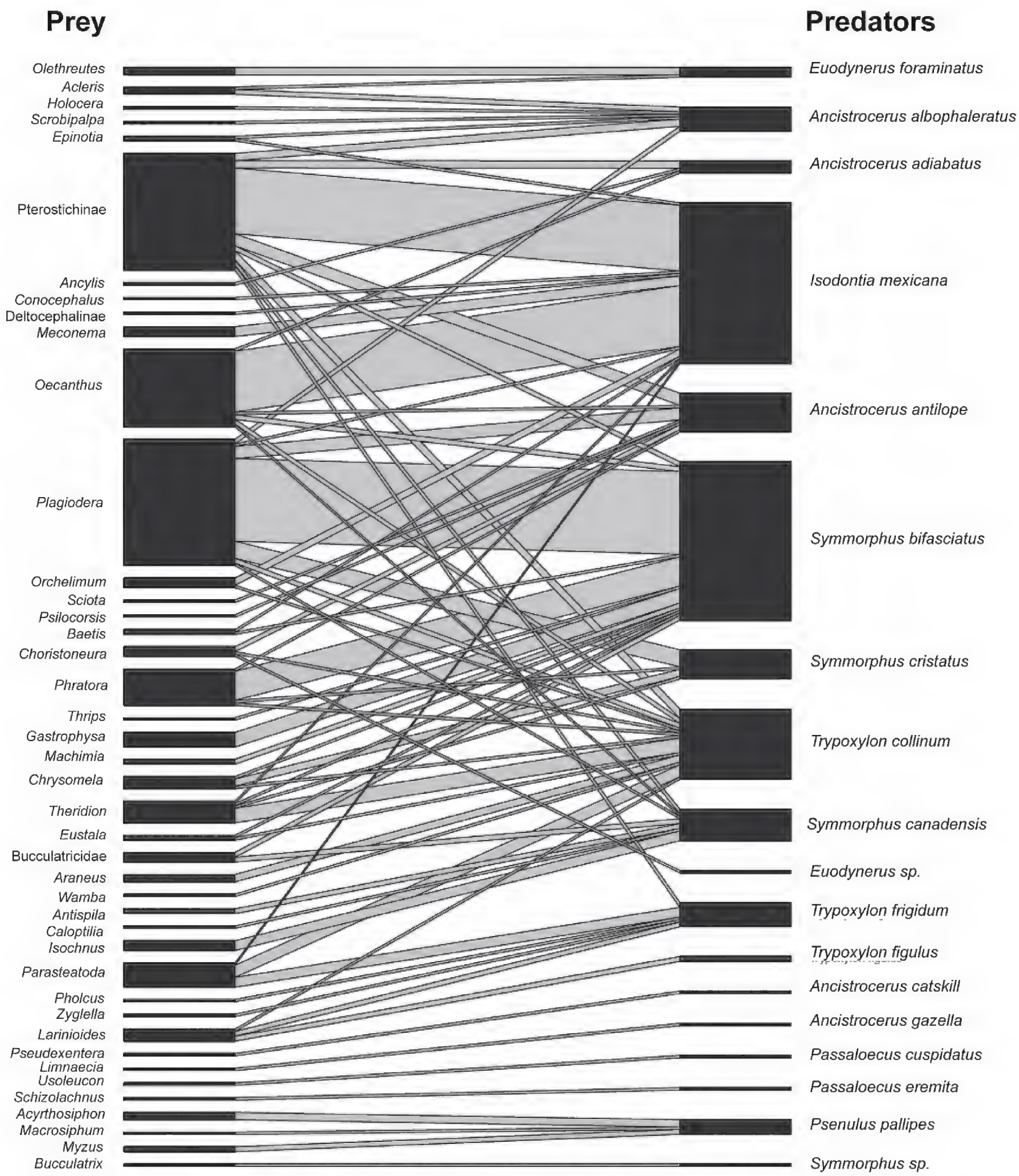

Figure 5. Bipartite network depicting prey and predators in trap nests in 2019 and 2020.

500 m radius of a site. Comparisons of bee and wasp species richness and abundance among landcover types (Table 4) did not reveal any significant patterns ($p > 0.05$) except for wasp abundance in 2019 (ANOVA, $p = 0.04$). However, post-hoc tests showed no significant differences (Tukey's Honest Significant Difference $p > 0.05$). While there were no differences in abundance or species richness, bee community composition differed among landcover types with *Megachile rotundata* most abundant in cropping landscapes,

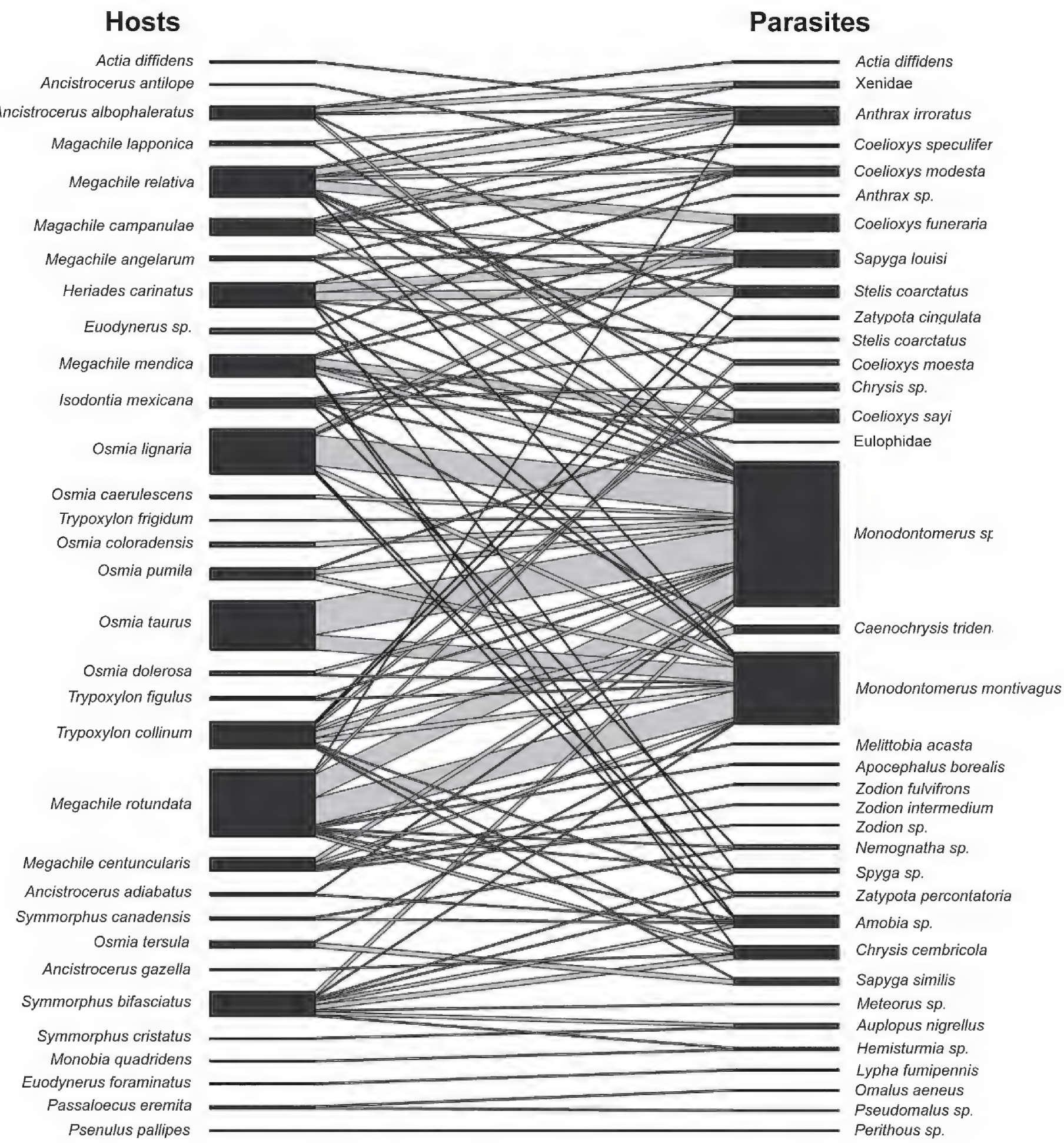

Figure 6. Bipartite network depicting parasites and hosts in trap nests in 2019 and 2020.

Table 4. Mean trap-nest occupancy, number of occupied and barcoded tubes, bee and wasp species richness and abundances (+/- SE) during 2019 and 2020, sorted by landcover designation. Landcover designation was determined in QGIS using the 2015 Land Cover of Canada dataset within a 500-metre radius circle around each site.

Designation	Year	Occupied Nests	Empty Nests	Total Nests	Occupied Tubes	Barcoded Tubes	Bee Richness	Bee Abundance	Wasp Richness	Wasp Abundance
Crop	2019	6	5	11	86	46	1.67 +/- 0.33	6 +/- 2.31	3 +/- 1.03	7 +/- 2.67
	2020	25	12	37	182	159	6.3 +/- 0.7	13.21 +/- 2.62	4.71 +/- 0.67	13 +/- 2.68
Forest	2019	4	2	6	19	14	3.33 +/- 2.4	9 +/- 7.55	1 +/- 0	1 +/- 0
	2020	20	21	41	104	97	5.1 +/- 0.76	8.6 +/- 1.72	5.05 +/- 0.66	9.45 +/- 1.7
Urban	2019	12	15	27	67	32	1.36 +/- 0.31	2.55 +/- 0.67	1.55 +/- 0.37	2 +/- 0.66
	2020	48	44	92	318	293	5.71 +/- 0.58	11.9 +/- 1.86	4.82 +/- 0.43	11.8 +/- 1.88
Total		**115**	**99**	**214**	**776**	**638**				

Megachile relativa most abundant in forested landscapes, and *Osmia lignaria* and *Megachile rotundata* most common in urban areas.

Discussion

Solitary cavity-nesting bees and wasps, and their natural enemies, provide important ecosystem services through their interactions (Ollerton et al. 2011; Hoffmann et al. 2018; Brock et al. 2021). This study investigated their distributions and interactions across Canada by pairing the augmented data collection power of community science with DNA barcoding. Community science often allows studies to gather samples from a far larger area than could have been accomplished otherwise (Henter et al. 2016; Steinke et al. 2017). Additionally, such projects provide a unique educational opportunity for participants (MacPhail and Colla 2020). Over the course of its five-year run the Bees@Schools program reached hundreds of students, teachers, and families. The results of the first two years are presented in this study and these already enabled us to expand known distribution ranges, gain new insights into foraging behaviour and discover novel interactions for cavity nesting bees and wasps, as well as insights into how local landcover might affect their nesting.

Metabarcoding studies usually deliver much larger datasets than observational approaches. However, as the balance of this section shows, they need careful evaluation. Therefore, species found with potential range expansions were compared to matching records on BOLD to ensure the existence of a reference specimen in a collection. In some cases, e.g. *Osmia taurus* and *Trypoxylon* cf. *nitidum*, some 100% barcode match reference specimens were sent to or photographed for taxonomic experts to corroborate their identities. Another key factor for successful metabarcoding is the quality and completeness of reference libraries (Meiklejohn et al. 2019; Pentinsaari et al. 2020). Extensive barcoding work in Canada resulted in the most comprehensive national reference libraries for terrestrial arthropods (deWaard et al. 2019; Steinke et al. 2022) and plant barcodes (Braukmann et al. 2017), and our study greatly benefitted from both.

Distribution ranges

Knowledge of species' ranges is essential to make informed management decisions and track changes over time (Jamieson et al. 2019). Although our sampling was not site-intensive, three non-native and seven native bee species were found in previously unrecorded provinces or areas. Whether these detections represent true range expansions or are the result of limitations of previous sampling is difficult to determine. While some may be due to low sampling intensity, species known only from western Canada such as *Megachile angelarum*, *Megachile snowi*, and *Osmia dolerosa* were all found in southern Ontario which has been intensively sampled by multiple working groups (MacIvor 2019). Likewise, *Coelioxys modesta* and *M. campanulæ* are considered eastern Canada species but we detected them in southern British Columbia. Sheffield and Heron (2019) provide a comprehensive checklist of bee species in British Columbia but do not include either species. Given that such guides were published very recently makes it unlikely that these species are new introductions; instead they

might have been overlooked by the use of methods other than trap-nesting. This is often the case for kleptoparasite species such as *C. modesta* that do not collect pollen for their offspring and are not as attracted to pan traps as pollen-foraging species (Wilson et al. 2008). While the native species *Megachile mendica* and *Osmia cærulescens* were found within provinces where they had previously been recorded, locations do suggest range expansions to the North.

We found *Chelostoma rapunculi*, previously only reported in Ontario, at one site in British Columbia and the introduced species *Hylæus pictipes* previously only reported from Ontario, was also found it in Manitoba. Finally, and most surprisingly, numerous samples included matches to *Osmia taurus* in British Columbia, Alberta, Manitoba, Ontario, and Prince Edward Island while it has previously only been seen in Ontario. The frequency and wide distribution of these records is particularly interesting because its recent progress throughout the United States and into Canada has been closely watched, with a rapid increase of *O. taurus* observations in the Mid-Atlantic United States since its first detection in 2002 (Le Croy et al. 2020). This species is thought to have been accidentally imported in place of the very similar-looking *Osmia cornifrons* that is used agriculturally (Gibbs et al. 2017; LeCroy et al. 2020). This finding is perhaps the most contentious because detecting it as far north as Churchill (Manitoba) represents a massive range expansion over a relatively short time. We therefore took reference specimens which were 100% matches to our barcodes to a taxonomic expert (Dr. Tom Onuferko, Canadian Museum of Nature), who confirmed their identity. Solitary cavity-nesting hymneoptera are uniquely positioned to be distributed to new areas quickly due to their nesting habits. While they might nest in shipping containers and be accidentally transported, there are also companies who intentionally ship cavity-nesting bee cocoons (e.g. *Osmia lignaria* and *Osmia cornifrons*).

We found four species of cavity-nesting wasps outside their historically reported ranges in Canada. Three of these are non-native species that had previously been found in Ontario or eastern Canada, but we additionally found them in British Columbia. These species had few detections meaning they might have only recently arrived on the west coast. *Trypoxylon nitidum* is native to South America (Scher and das Graças Pompolo 2003), although a few records are available from southern Ontario. In addition to Ontario, we found *Trypoxylon* cf. *nitidum* in British Columbia, Alberta, and Manitoba. At this point, further investigation into this is required since it might represent a closely related but undocumented species or a significant range expansion.

Foraging

Cavity-nesting bee foraging

The overall plant-pollinator network showed a community with low nestedness. This indicates high beta diversity and dissimilar species assemblages among sites (Wright and Reeves 1992; Dormann et al. 2009). Although low nestedness is often associated with low network stability (Gresty et al. 2018), in this case it is likely caused by differences between sites given that this network spans collections across Canada. Overall network connectance (0.59) indicates that 59% of the potential interactions were realized (Dormann et al. 2009; Rivera-Hutinel et al. 2012). A higher level of connectance suggests high network stability since

there is some redundancy (Gresty et al. 2018) and indeed the dataset includes a high number of species with many single links.

Asteraceae (20%), Fabaceae (13%), and Rosaceae (13%) were the most abundant plant families found in tube nests occupied by cavity nesting bees. As Asteraceae and Fabaceae are two of the largest plant families (Panero and Crozier 2016), their high level of detection is not surprising. Our plant-pollinator bipartite networks revealed largely pollen consumption patterns consistent with generalist foragers. For most bee species detected, this agrees with historical records classifying them as polylectic. However, three species classified as oligolectic (*Chelostoma rapunculi*, *Megachile lapponica*, *Megachile pugnata*) appeared to forage on plant genera and families outside their known hosts. Some of these observations coincided with multiple occupancy of nests which could explain some of the additional plant genera detected, however they cannot explain missing known specialist pollen sources in two of the species. Prior work showed that plant-pollinator networks build with observational data can differ considerably from those that use pollen metabarcoding, to the extent that species considered oligolectic through observation, were reclassified as polylectic based on metabarcoding data (Arstingstall et al. 2021). Some of these additional observations might be explained by instances when a bee inadvertently collected various pollen species during one flower visit, simply because the pollen was deposited there through wind or other flower visitors. As plant-centred visual surveys tend to skew the true number of specialist bee species (Bosch et al. 2009), it is therefore possible that some of the bee species generally considered oligolectic will use alternative pollen sources when needed. However, since specialists are defined by the pollen they collect, it could also be that detections of nectar within bee bread are contributing to increased plant diversity of foraging sources. Determination of the exact reason for these interactions is outside the scope of this study, but it is likely to be a combination of multiple nesting, nectar detection, and sensitive detection through DNA barcoding.

Cavity-nesting wasp prey

Solitary wasps in the families Pompilidae, Sphecidae, and Vespidae are considered prey specialists, while the Crabronidae are more generalists (Brock et al. 2021). Of the 23 species of cavity-nesting wasps that were analyzed with our bipartite networks, 10 were found with prey outside their recorded choices. As a member of the genus *Trypoxylon*, *T.* cf. *nitidum* is historically known as a spider-collecting wasp. In addition to spiders in the genera Araneidae and Theridiidae, *T.* cf. *nitidum* collected beetle and moth larvae as well as grasshoppers. *Isodontia mexicana* is likewise well-known to collect grasshoppers but was also noted to collect beetles, moth larvae, and spiders. Multiple species of *Ancistrocerus* provisioned beetle larvae or grasshoppers, in addition to their recorded preference for moth larvae. Some of these detections are likely explained by nest-sharing, however they might also represent a less preferred but still acceptable prey. Many of these historical predator-prey records are from observational studies from the 1900s, so further investigation using modern methods should be conducted.

Other Interactions

Interspecific nest sharing

A quarter of all nesting tubes contained two or more solitary cavity-nesting predatory wasp and/or pollinating bee species (Suppl. material 1: table S5). This pattern might occur, even in the presence of sufficient empty nests, if the first species abandons their nest early and another takes up occupancy or if the nest is usurped (Černá et al. 2013). Usurpation occurs when a female takes over the nest of another female that is in the process of being built (Černá et al. 2013). Multiple occupancies have been reported in other studies including interspecific usurpation in *Megachile inermis* and *Megachile relativa* nests where each species was both usurped and usurper (Strickler et al. 1996). From their trap-nesting study, O'Neill and O'Neill (2018) found 3% of nests in which the first species of bee or wasp was superseded by another. They report instances of either two bees or wasps or a combination of the two co-habiting. It is possible that the high proportion of multiple occupancies found in this study was due to increased detection sensitivity through DNA barcoding compared to previous studies in which occupants were allowed to hatch out into contained spaces. In those cases, any pupae which failed to hatch would have been very difficult to identify. Additionally, hosts or prey would have been consumed, confounding determination of who the parasite or predator was. For these reasons, metabarcoding represents a superior approach to investigating interspecific nest sharing. Our results together with prior studies provide rationale for the large number of nests found with two species; however, they do not address the cases (N = 35) where three or four species were found together. Whilst it is possible that these represent multiple abandonments or usurpations, this remains an interesting area for future studies to investigate.

Parasitism

Half of all occupied sites and a quarter of occupied nesting tubes were parasitized. Previous studies report parasitism rates of 13% of nests (Loyola and Martins 2006) or 5% (Klein et al. 2006) and 7% (Steckel et al. 2014) of brood cells. Once again, those studies relied on hatching out occupants to observe parasitism that could contribute to the higher rates seen in this study. It has been suggested that trap nests could increase parasitism rates (Moenen 2012; MacIvor and Packer 2015), attributing this mainly to the high nesting densities that provide excellent host sources for parasites (Harris et al. 2021). Trap nests encourage different species to nest in proximity through differently sized tubes, which may also allow parasites to cross between species.

We found new host records for ten parasitic species which is not surprising given the paucity of recent host records for Canada. *Cænochrysis tridens* parasitized *Trypoxylon* cf. *nitidum*, although its host is reported to be *Trypoxylon politum* in Pennsylvania (Conrow et al. 2016). *T. politum* is not found as far north or west as *C. tridens* and so it likely uses an alternative host. *Symmorphus canadensis* has been reported as a host for *Chrysis cembricola* (Krombein and Hurd 1979 but since then no further hosts have been observed. We found it to parasitize five species of bees and wasps, including

Symmorphus bifasciatus. Four *Coelioxys* species were found with previously unrecorded hosts. As *Coelioxys* are known parasites of *Megachile* bees (Scott et al. 2000), new *Megachile* sp. records are not surprising but we also found it parasitizing on three species of wasps. *Stelis coarctatus* is known to parasitize *Heriades carinatus*, though Gibbs et al. (2017) notes that it has a broad host range, meaning the additional hosts represent expansion to this list. Similarly, *Amobia* sp., a dipteran parasite, only known from Vepid and Sphecid wasps (Spofford et al. 1989) and *Hylæus* (Marinho and Vivallo 2020) was found parasitizing on Megachilidae. For *Sapyga louisi* and *Sapyga similis* we were unable to find any prior host information which means our study provided first records.

Landcover

There are many reports of anthropogenic modification of landscapes affecting species and their interactions, including urbanisation (Hooper et al. 2005; Chace and Walsh 2006; McKinney 2006; Winfree et al. 2009). Urban landscapes have been observed to support reduced pollinator biodiversity (Elmqvist et al. 2003; Potts et al. 2010; Sánchez-Bayo and Wyckhuys 2019), with impacts largely based on the degree of urbanization and the type of bee (Wenzel et al. 2020). Some studies have found that urban areas positively influence solitary bee populations and areas of medium urbanization generally support pollinator populations, especially when compared to intensive agricultural areas (Fortel et al. 2014). Compared to ground-nesting bees, cavity-nesting bees tend to fare better in the face of urbanization since they do not depend on exposed soil for nesting (Wenzel et al. 2020). With these previous findings in mind, the lack of significant results when comparing bee and wasp species richness and abundance between cropping, forest, and urban landcovers is not surprising. Sampling sites were all located either on school property or on a teacher's property during COVID-19 lockdown in 2020. While these sites were distinguishable as being predominantly agricultural, forest, or urban, it is unlikely that many of them would be considered intensely agricultural or intensely urban landcovers. This might have reduced the signal of abundance or species richness differences among landcover types. The observed significant differences in wasp species richness and abundance in 2019 forests is likely an effect of lower sample sizes during this pilot year. Likewise, the significantly higher species richness and abundance found in 2020 compared to 2019 is likely due to increased sampling efforts in 2020. These trends can be examined further once additional data are collected from further years of sampling.

Conclusions

Our results greatly expand our knowledge of parasite-host and predator-prey interactions in the solitary cavity-nesting Hymenoptera. A community science approach enabled extensive field work and provided educational opportunities for some 5000 students across Canada. Metabarcoding trap nest inhabitants has revealed interactions not previously observed by the great studies of the 20th century (Krombein 1967; Krombein and Hurd 1979). Natural history observations for many of these species have not been pursued using updated

technology, and great benefits can continue to be had from doing so. Solitary cavity-nesting bees and wasps provide essential pollination and predation services. These benefits are fueled by their interactions that are notoriously difficult to study. Further knowledge of these interactions can help advise conservation policies meant to support these beneficial insects and the ecosystem services they provide. This study shows a potential approach to more detailed study.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: DS, SH, NER. Data curation: TB, SH. Formal analysis: DS, TB, SH. Funding acquisition: NER, DS. Investigation: KC, SH. Methodology: TB, SH. Project administration: DS. Supervision: NER, DS. Validation: DS. Visualization: DS, SH. Writing - original draft: SH. Writing - review and editing: KC, NER, DS, TB.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary document

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